IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:	Confirmation No.: 2792
CHAN *et al.*	Art Unit: 1638
Appl. No.: 10/520,033	Examiner: Vinod Kumar
§ 371 Date: December 30, 2004	Atty. Docket: 2510.0040000/JAG/SAC
For: **Transcription Factor Gene Induced by Water Deficit Conditions And Abscisic Acid From Helianthus Annuus, Promoter And Transgenic Plants**	

Declaration Under 37 C.F.R. § 1.132 of Federico Trucco, Ph.D.

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

I, Federico Trucco, Ph.D., declare and state as follows:

1. I received my education at the University of Illinois, Urbana-Champaign. A copy of my *curriculum vitae* is attached as Exhibit A.

2. I am currently employed at INDEAR S.A., a subsidiary of Bioceres S.A., the assignee of the above-captioned application. I hold the position of Head of Development. My work involves the evaluation of plant transgenic events at the different stages of the development pipeline, characterizing new technologies in terms of agronomic performance and biosafety.

3. I am familiar with the above-identified application and pending claims as well as the February 22, 2008 Office Action.

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4. I understand that the present claims 10, 14, 15, 17, 18 and 21 are directed to a transgenic plant, plant seed, plant host cell that have been stably transformed with and express the *Hahb-4* gene as well as a method for producing a water stress tolerant transgenic plant by stably transforming and expressing *Hahb-4* in a plant cell or cell culture and regenerating the plant cell or cell culture into a plant.

5. I understand that the claims have been rejected in the Office Action for, among other things, being obvious in view of Gago *et al.*, *Plant, Cell and Environment* 25:633-640 (2002) (hereinafter "Gago") and U.S. Patent No. 6,265,638 (Bidney *et al.*) (hereinafter "Bidney"). I have reviewed both of these publications.

6. In my view, a "person of ordinary skill in the art" with respect to the above-identified patent application would be a person having at least graduate level training and experience in the field of plant molecular biology.

7. It is my opinion that based on the publications cited by the Examiner and the knowledge in the art at the time the above-captioned application was filed, one of ordinary skill in the art would not have predicted that the expression of *Hahb-4* in transgenic plants would result in the development of a drought tolerant phenotype in these plants. Specifically, it was known in the art at the time the application was filed that transcription factors, including those induced by water stress, regulated a wide variety of target genes, many of which may not have been involved in drought tolerance. *See, e.g.*, Ingram and Bartels, *Annu. Rev. Plant Physiol. Plant. Mol. Biol. 47*:377-403 (1996) (Exhibit B) and Shinozaki and Yamaguchi-Shinozaki, *Plant Physiol. 115*:327-334 (1997) (Exhibit C). Furthermore, even if a transcription factor regulated a particular gene

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involved in drought tolerance, post-transcriptional modification to the transcription factor could have been required in order to induce expression of the downstream target gene. Liu *et al.*, *The Plant Cell 10*:1391-1406 (1998) at page 1402 (Exhibit D). Thus, if post-transcriptional modifications of the transcription factor were required for the transcription factor's activity, expression of the transcription factor in a non-native host would not have been expected to provide drought resistance. One such example is the *Arabidopsis* transcription factor *DREB2*, which is induced rapidly by water stress. However, the induction of *DREB2* alone was not sufficient for drought tolerance, suggesting that *DREB2* proteins required posttranslational activation. *See* Liu *et al.*, *The Plant Cell 10*:1391-1406 (1998) at page 1402 and Nakashima and Yamaguchi-Shinozaki, *JARQ 39*:221-229 at page 223 (citing Liu *et al.*) (Exhibit E). Thus, at the time the application was filed, it is my opinion that one of ordinary skill in the art could not have predicted that a transcription factor that was induced under water stress conditions would correlate with the ability of the transcription factor to confer drought tolerance. Even today, as pointed out in Century *et al.*, the use of transcription factors to induce drought tolerance is challenging--"[t]he need for improved abiotic stress tolerance in crop plants is great, but engineering these traits is particularly challenging because multiple complex pathways are involved in controlling the native stress responses in plants." Century *et al.*, *Plant Physiol. 147*:20-29 (2008).

8. It is also my opinion that despite previous failure by others to produce transgenic plants that are drought tolerant, the present invention provides multiple species of transgenic plants that are unexpectedly drought tolerant and fulfill a long-felt need.

9. Growing in their natural environment, plants often encounter unfavorable environmental conditions that interrupt normal plant growth and productivity. Of these unfavorable environmental conditions, drought is one of the most devastating conditions for crops. Climate change and population growth create for crop researchers one of the greatest challenges facing humanity today: the growth of productive crops in water-limited environments. *See* Pimentel *et al.*, *BioScience* 47:97-106 (1997) (Exhibit F). From a plant breeding standpoint, drought tolerance follows quantitative genetics. As is the case for other highly polygenic traits, conventional methods of crop improvement can provide only marginal gains. Thus, rapid progress in this area depends on our ability to effectively transform crops to achieve phenotypes beyond the boundaries of a crop's genetic variation.

10. As described in the specification, *Hahb-4* is a member of the Hd-Zip gene family, which includes the *Arabidopsis* genes, *Athb-7* and *Athb-12*. The HAHB-4 protein shares extensive homology in its homeodomain with ATHB-7 and ATHB-12. In addition, HAHB-4 shares with ATHB-7 and ATHB-12 the lack of an acidic domain that is present in other members of the Hd-Zip protein family. *See* specification at page 16, lines 7-19. Like *Hahb-4*, both *Athb-7* and *Athb-12* are induced in response to drought or water stress. *See* Soderman *et al.*, *Plant J. 10*:375-381 (1996) (Exhibit G), Lee and Chun, *Plant Mol. Biol. 37*:377-384 (1998) (Exhibit H) and Olsson *et al.*, *Plant Mol. Biol. 55*:663-677 (2004) (Exhibit I). However, despite this induction during water stress, *Athb-7* and *Athb-12* have not been shown to confer drought tolerance to transgenic plants. *See* Hjellström *et al.*, *Plant Cell and Environment 26*:1127-1136 (2003) at page 1132 (Exhibit J) and Olsson *et al.*, *Plant Mol. Biol. 55*:663-677 (2004) at page 671.

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11. In contrast, experiments described in the present application show that the *Arabidopsis thaliana* transgenic plant carrying the *Hahb-4* gene germinates more rapidly as compared to the non-transformed control plants under water-stress conditions. *See* Figure 8. Remarkably, the transgenic plants subjected to water stress also reached a stem height similar to the stem height of the same transgenic plant grown under normal water conditions as well as a stem height that was equivalent to 85% of the height of a control plant under normal water conditions. *See* Figure 9. Thus, these results indicate that the water stress does not affect the stem growth in the transgenic plants of the invention and not only are the transgenic plants tolerant to the water stress but they grow normally in these lack-of water conditions. *See* specification at page 21, lines 4-22.

12. Additional experiments showed that when the transgenic plants were subjected to water stress at various developmental stages including the adult, vegetative, germination, and reproductive stages, the transgenic plants were more tolerant and resistant to water stress than the control plants. *See* specification at page 23, line 22 through page 24, line 3; page 25, lines 12-19 and Figure 17.

13. Furthermore, as the specification points out, despite the fact that the transgenic plants exhibit several phenotypic differences such as slightly shorter stems and more rounded and less elongated leaves, these phenotypic changes remarkably do not affect the production and germination of the plants. On the contrary, the production of seeds of the transgenic plants is higher than the production of the non-transformed plants under water-stress conditions. *See* specification at page 24, line 20 through page 26, line 12.

14. In addition to providing a dicot transgenic plant (*Arabidopsis*) that is drought tolerant, post-filing greenhouse studies have also demonstrated that *Hahb-4* can be expressed in and produce a drought resistant phenotype in two different monocots. These data show that the expression of *Hahb-4* in wheat and maize results in a drought tolerant transgenic plant. *See* attached Exhibit K and L. Exhibit K demonstrates that two separate wheat transgenic lines expressing *Hahb-4* show an average 75% increase in yield compared to wild-type wheat plants subjected to the same water stress conditions. Exhibit L demonstrates that three separate maize transgenic lines expressing *Hahb-4* retain on average approximately 50% of their total leaf area measured prior to water stress induction, compared to the non-transgenic control, which only retained 24% of the original leaf area. Thus, the expression of *Hahb-4* in various species of plants has unexpectedly met a long-felt need of providing productive agronomically valued transgenic plants.

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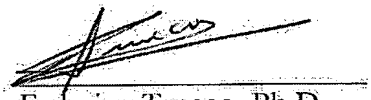
15. I hereby declare that all statements made herein of my own knowledge are true and that all statement made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the present patent application or any patent issued thereon.

Respectfully submitted,



Federico Trucco, Ph.D.

Date: 08-22-2008

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